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(54) Title: **AUTOLOGOUS THROMBIN**

(57) Abstract: A sterile method for preparing stable thrombin component from a single donor's plasma in which the thrombin component and the clotting and adhesive proteins component are harvested simultaneously from the same donor plasma in less than one hour. The combined components provide an improved biological hemostatic agent and tissue sealant by virtue of its freedom from the risk of contaminating viruses or bacteria from allogenic human or bovine blood sources. The thrombin provides polymerization of the clotting and adhesive proteins in less than five seconds, and is sufficiently stable to provide that fast clotting over a six hour period. Further, the clotting times can be predictably lengthened by diluting the thrombin with saline.

AUTOLOGOUS THROMBIN

Technical Field

5 The following invention relates generally to the preparation of a high specific activity thrombin enzyme from a given unit of plasma, which is sufficiently stable that it provides rapid clotting of a fibrinogen-rich solution of clotting and adhesive proteins for more than six hours when held at room temperature or lower.

Background Art

10 Formulation of a fibrin sealant mimics the last step of the coagulation cascade wherein the enzyme thrombin cleaves fibrinogen which is then cross-linked into a semi-rigid or flexible fibrin clot. This fibrin clot adheres to wound sites, forming a barrier to fluid leaks and generates adhesion between tissues, while providing hemostatic and healing properties to the treated site.

15 Presently marketed, applicant's CryoSeal™ system is a device which harvests cryoprecipitated, concentrated clotting and adhesive proteins, including fibrinogen and Factor XIII from a donor's plasma in approximately one hour. The one hour cryoprecipitation harvesting, provided by the CryoSeal™ system, compared to the 1 to 2 day cryoprecipitation process routinely practiced in Blood Banks, means that CryoSeal™ harvesting of clotting and adhesive proteins can occur right in the
20 perioperative theater with the patient close by, thereby avoiding the need to initiate the process days in advance.

These CryoSeal™ harvested clotting and adhesive proteins, when combined with bovine or human thrombin, forms a biological glue useful for surgical hemostasis and tissue adhesion. Commercially available thrombin, however, is
25 generally sourced from bovine or human plasma pools, so the patient would still be at risk of negative immune reactions or contamination by infectious blood born viruses and, possibly Crutzfeld-Jacobs Disease (CJD) or new variants of CJD (NVCJD). An advantage of the CryoSeal™ cryoprecipitation invention is that the harvested clotting and adhesive proteins sourced from the patient's own blood
30 eliminates the risk of contamination by infectious blood-borne disease when these

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	Rose, et al.	4,627,879	December 9, 1986
	Redl, et al.	4,631,055	December 23, 1986
	Sakamoto, et al.	4,655,211	April 7, 1987
5	Silbering, et al.	4,696,812	September 29, 1987
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	Koizumi, et al.	4,734,261	March 29, 1988
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	Saferstein, et al.	4,752,466	June 21, 1988
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	Skorka, et al.	4,826,048	May 2, 1989
	Davis	4,842,581	June 27, 1989
	Miller, et al.	4,874,368	October 17, 1989
	Avoy	4,902,281	February 20, 1990
15	Seelich	4,909,251	March 20, 1990
	Tanaka, et al.	4,923,815	May 8, 1990
	Silbering, et al.	4,965,203	October 23, 1990
	Capozzi, et al.	4,978,336	December 18, 1990
	Wolf, et al.	4,979,942	December 25, 1990
20	L'Hermite, et al.	4,987,336	January 22, 1991
	La Duca	5,089,415	February 18, 1992
	Kotitschke, et al.	5,099,003	March 24, 1992
	Wolf, et al.	5,104,375	April 14, 1992
	Capozzi, et al.	5,116,315	May 26, 1992
25	Nishimaki, et al.	5,130,244	July 14, 1992
	Kraus, et al.	5,143,838	September 1, 1992
	Crowley, et al.	5,151,355	September 29, 1992
	Knighton	5,165,938	November 24, 1992
	Galanakis	5,185,001	February 9, 1993
30	Morse, et al.	5,219,328	June 15, 1993
	Fischer	5,290,259	March 1, 1994
	Sierra, et al.	5,290,552	March 1, 1994
	Michalski, et al.	5,304,372	April 19, 1994
	Fischer	5,328,462	July 12, 1994
35	Lonneman, et al.	5,368,563	November 29, 1994
	Linnau	5,393,666	February 28, 1995
	Epstein	5,405,607	April 11, 1995
	Marx	5,411,885	May 2, 1995
	Kikuchi, et al.	5,443,959	August 22, 1995
40	Miller, et al.	5,474,540	December 12, 1995
	Broly, et al.	5,474,770	December 12, 1995
	Weis-Fogh, et al.	5,480,378	January 2, 1996
	Proba, et al.	5,506,127	April 9, 1996
	Cochrum	5,510,102	April 23, 1996
45	Antanavich, et al.	5,585,007	December 17, 1996
	Pines, et al.	5,605,887	February 25, 1997
	Cochrum	5,614,204	March 25, 1997
	Marx	5,631,019	May 20, 1997
	Hirsh, et al.	5,643,192	July 1, 1997

before they can perform their function. A surgeon utilizing the Hirsh fibrin glue would be required to arrange his surgery so that the hemostasis and tissue sealing intended for treatment with the Hirsh fibrin glue would occur within the 4 minute window where the clotting time was less than 5 seconds, making the Hirsh invention totally impractical for most surgeries which predominantly require rapid hemostasis and tissue adhesion throughout the surgery, the time span of which could extend to six hours.

The following prior art reflects the state of the art of which applicant is aware and is included herewith to discharge applicant's acknowledged duty to disclose relevant prior art. It is stipulated, however, that none of these references teach singly nor render obvious when considered in any conceivable combination the nexus of the instant invention as disclosed in greater detail hereinafter and as particularly claimed.

<u>U.S. PATENT DOCUMENTS</u>		
	<u>PATENT NO.</u>	<u>ISSUE DATE</u>
15 <u>INVENTOR</u>		
Pumphrey	713,017	November 4, 1902
Mobley	1,614,532	January 18, 1927
Ferry, et al.	2,533,004	December 5, 1950
Wahlin	2,747,936	May 29, 1956
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Meurer, et al.	3,269,389	August 30, 1966
Venus, Jr.	3,416,737	December 17, 1968
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Creighton, et al.	3,828,980	August 13, 1974
Green	3,942,725	March 9, 1976
Polnauer, deceased, et al.	3,945,574	March 23, 1976
Speer	4,040,420	August 9, 1977
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Kozam, et al.	4,109,653	August 29, 1978
Sugitachi, et al.	4,265,233	May 5, 1981
Schwarz, et al.	4,298,598	November 3, 1981
Redl, et al.	4,359,049	November 16, 1982
35 Schwarz, et al.	4,362,567	December 7, 1982
Altshuler	4,363,319	December 14, 1982
Schneider	4,374,830	February 22, 1983
Schwarz, et al.	4,377,572	March 22, 1983
Schwarz, et al.	4,414,976	November 15, 1983
40 Stroetmann	4,427,650	January 24, 1984
Stroetmann	4,427,651	January 24, 1984
Stroetmann	4,442,655	April 17, 1984

clotting and adhesive proteins are topically applied to the patient's surgical wound sites.

It has long been understood, however, that the safest condition for a surgical patient would result from a two component biological sealant preparation in which the thrombin component would be harvested from the same donor in which the clotting and adhesive protein component was harvested - forming a fully autologous biological sealant or glue.

The concept of utilizing thrombin and/or fibrinogen sourced from the patient in a medical procedure performed on that patient is not novel and was first described by Andrianova in 1974. Some twenty years later, Cederholm-Williams PCT Patent (WO94/00566 - 6 January 1994) describes an improved fibrin glue in which the thrombin component, which required thirteen steps, including centrifugation, and separation of intermediate precipitates and adjusting the ionic strength of the blood and pH of the plasma to prepare, would be combined with a fibrinogen component also sourced from the plasma of the same donor. However, these many preparation steps are so time consuming they become impractical for use in the perioperative theater where processing times should be less than one hour.

Three years later, in 1997, Hirsh, et al. (U.S. Patent No. 5,643,192) follows Cederholm-Williams by teaching another method of preparing fibrin glue in which both the fibrinogen and thrombin components of a fibrin glue are sourced from the same donor's plasma. The Hirsh patent describes a method of preparing thrombin in which most of the fibrinogen in the plasma is first precipitated and removed to prepare a supernatant and then clotting the residual fibrinogen in the supernatant which is different and simpler than the method taught by Cederholm-Williams, but does not result in a commercially useful thrombin in that (see figure 1 of Hirsh, et al.) the thrombin provides clotting speeds of five seconds or less for only 4 minutes, and less than 10 seconds for only 47 minutes.

These clotting speeds are unsuitable to the needs of surgeons who could not plan their entire surgeries around the limitations of the Hirsh, et al. fibrin glue.

Surgeons predominately require a fast acting clotting time (< 5 seconds) for hemostasis and tissue sealing or adhesion. Slow clotting biological glues (> 5 seconds) will often be transported away from the wound site by oozing and bleeding

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	Edwardson, et al.	5,750,657	May 12, 1998
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	Szent-Györgyi, et al.	CH	259,254	June 1, 1949
10	The Trustees of Columbia University in the City of New York	WIPO	WO 86/01814	March 27, 1986
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	Octapharma AG	EP	0 534 178 A2	March 31, 1993
	Cryolife, Inc.	WIPO	WO 93/19805	October 14, 1993
	Cederholm-Williams, et al.	WIPO	WO 94/00566	January 6, 1994
	E.R. Squibb & Sons	EP	0 592 242 A1	April 13, 1994
25	Plasmaseal Corporation	WIPO	WO 96/17871	June 13, 1996

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10 The other prior art listed above, not all of which are specifically discussed catalog the prior art of which the applicant is aware. These undiscussed references diverge even more starkly from the instant invention specifically distinguished below.

Disclosure of Invention

15 The instant invention addresses the long felt need for a simple, practical, fast method of preparing stable human thrombin from a donor's blood, which will provide fast clots (< 5 seconds) throughout a lengthy surgery (e.g. six hours) to combine with the clotting and adhesive proteins harvested and concentrated from the same unit of blood to form a biological sealant with no patient exposure to microbial or possible CJD or NVCJD contaminations. Previous works in the field (Hirsch, et al.) exemplified a thrombin with minimal stability in that the thrombin achieved rapid clotting of fibrinogen (i.e., less than 5 seconds) during only a very narrow four to five minute time period, or required so many steps and elapsed time it would not be suitable for perioperative preparation, both totally impractical for the broad range of surgeries.

25 The present invention provides a stable thrombin enzyme which can cause precise, repeatable fast or slow polymerization of clotting and adhesive proteins over a duration of up to six hours - throughout even a long surgery. Further, the use of clotting and adhesive proteins and thrombin all sourced from a single donor will eliminate various disease risks posed from the use of commercial fibrin glues where the fibrinogen is sourced from plasma pooled from thousands of donors and the thrombin is either sourced from a similar pool of human plasma or of bovine origin. The speed and simplicity of the production of stable thrombin by use of this invention allows it to be prepared just prior to or during operative procedures and it will provide fast clotting throughout even the longest surgeries. The thrombin produced by this invention can be diluted in saline, water and a dilute CaCl_2

30

solution (e.g. 125 mM CaCl_2) to provide precise, slower clotting times thereby allowing any preferred time from less than five seconds to longer than 2 minutes.

The procedure of the invention is preferably comprised of three steps, the first two of which should preferably occur at the same time:

- 5 1. Preparing a fraction enriched in prothrombin by use of Ethanol to substantially enhance the concentration of prothrombin and at the same time remove or denature naturally occurring ingredients within plasma, such as Fibrinogen and Antithrombin III which can bind to, block, interfere with or inhibit prothrombin or its subsequent activation to long-term functional thrombin.
- 10 2. Adding calcium ions to the enriched prothrombin solution and briefly agitating the solution to convert the pro-thrombin to stable, long term thrombin.
3. Expressing the thrombin solution through a filter to remove particulate matter which would prevent spraying the thrombin through a small orifice or expressing the thrombin through a thin tube onto a wound site.

15

Industrial Applicability

The industrial applicability of this invention shall be demonstrated through discussion of the following objects of the invention.

Accordingly, it is a primary object of the present invention to provide a new and novel apparatus and method to derive fast acting, stable autologous thrombin
20 from the donor's plasma.

It is a further object of the present invention to provide thrombin as characterized above which has a shelf life longer than most associated surgical procedures.

It is a further object of the present invention to provide thrombin as
25 characterized above in which the clotting time can be predictably lengthened at will through dilution with saline.

It is a further object of the present invention to provide thrombin as characterized above which has simple preparatory procedures.

It is a further object of the present invention to provide a method for
30 producing thrombin as characterized above which has a process time in as little as thirty minutes, up to seventy-five minutes.

It is a further object of the present invention to provide thrombin which can be sprayed through small orifices or expressed through thin tubes.

Viewed from a first vantage point it is the object of the present invention to provide a novel and practical method for producing stable human thrombin from a prothrombin fraction which has been substantially enriched by ethanol fractionation to increase the prothrombin concentration and at the same time remove contaminating proteins. The addition of calcium chloride (CaCl_2) to the enriched prothrombin converts prothrombin to thrombin. From the same sole donor plasma, clotting and adhesive proteins are simultaneously obtained by other means to comprise the second component necessary for the autologous biological sealant.

Viewed from a second vantage point, it is an object of the present invention to provide a method for generating autologous thrombin from a patient, the steps including: obtaining a blood product from the patient; sequestering plasma from the product; enriching the prothrombin in a plasma fraction; converting the prothrombin to thrombin, and filtering particulate from the thrombin.

Viewed from a third vantage point, it is an object of the present invention to provide a method for producing autologous thrombin which is stable for more than fifteen minutes, the steps including: sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

Viewed from a fourth vantage point, it is an object of the present invention to provide an autologous thrombin which provides fast clotting in less than five seconds for more than fifteen minutes.

Viewed from a fifth vantage point, it is an object of the present invention to provide a composition for extracting thrombin from plasma consisting essentially of: Plasma; Ethanol (ETOH); CaCl_2 .

Viewed from a sixth vantage point, it is an object of the present invention to provide a method for preparing thrombin comprising: obtaining plasma; adding ETOH and CaCl_2 to the plasma, forming a composition; agitating the composition; incubating the composition in a static or rocking mode; filtering the composition of particulate, thereby passing the thrombin through the filter.

Viewed from a seventh vantage point, it is an object of the present invention to provide a device for preparing thrombin from plasma, comprising: a reaction

chamber having a solution of CaCl_2 and ETOH therein; means for admitting plasma into the reaction chamber; thrombin receiving syringe coupled to the reaction chamber to receive the thrombin; and a filter located between the reaction chamber and the thrombin receiving syringe.

5 Viewed from an eighth vantage point, it is an object of the present invention to provide an autologous biological glue processing device, comprising, in combination: a thrombin processing means, a clotting and adhesive proteins processing means operatively coupled to the thrombin processing means, means for receiving plasma via the operative coupling for subsequent conversion of the
10 plasma to, respectively thrombin and clotting and adhesive proteins.

The present invention provides a method and apparatus that produces thrombin which is sufficiently stable that it can provide less-than-5-second clots for up to six hours, substantially more stable than demonstrated in all prior art. Further, the clot time can be modified at will through dilution with saline.

15 The present invention further provides an efficient method of preparation. Improved cryoprecipitation of clotting and adhesive proteins through the CryoSeal™ invention requires less than one hour. In this same time frame, the autologous human thrombin component can be manufactured with minimal materials and methods from the same source plasma. Both of the biological
20 components of the biological glue are easily combined in a surgical setting, administered to the very same donor patient, and the resultant clotting provides hemostasis or tissue adhesion at the wound site.

The present invention additionally provides a method for sterile production of both components of the biological glue. The improved sterile manufacturing
25 described herein provides a final product that is essentially free of contamination by non autologous microbes.

These and other objects will be made manifest when considering the following detailed specification when taken in conjunction with the appended drawing figures.

30

Brief Description Of Drawings

Figures 1A and 1B are perspective views of apparatuses for sequestering prothrombin from plasma, processing the prothrombin into thrombin and taking

the plasma not relegated towards the prothrombin and extracting clotting and adhesive proteins therefrom.

Figures 2A and 2B are plan views of the thrombin processing sets removed from the processing sets that extracts clotting and adhesive proteins.

5 Figures 3A and 3B are perspective views of the interior of the thrombin processing cases with the thrombin syringe shown in figures 2A and 2B removed therefrom.

Figures 4A and 4B are perspective views of the thrombin cases upper halves.

Figures 5A and 5B are perspective views of the thrombin cases lower halves.

10 Figures 6A and 6B are exploded parts views of the reaction chamber 26 shown in figures 3A and 3B along with the valving structure at opposed ends thereof.

Figures 7A and 7B are sectional views of the reaction chambers and valving structures depicted in figures 6A and 6B.

15 Figures 8A and 8B are detail of construction of that which is shown in figures 7A and 7B.

Figures 9A and 9B are exploded parts view of filter alternatives used in figures 3A and 3B.

Figure 10 is a perspective view of that which is shown in figure 9.

20 Figure 11 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations.

Figure 12 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations at different CaCl_2 concentrations.

25 Figure 13 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored on ice.

Figure 14 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored at room temperature.

Figure 15 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored on ice.

Figure 16 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored at room temperature.

Best Mode(s) for Carrying Out the Invention

Referring to the drawings, wherein like elements denote like parts throughout, reference numeral 10 is directed to the processing set according to the present invention and shown in figures 1A and 1B.

In its essence, the processing set 10 includes a fluid receiving system 20 which communicates with both a thrombin processing unit 40 and a clotting and adhesive proteins processing unit 60.

More particularly, viewing both figures 1A and 1B, the fluid receiving system 20 includes an inlet 2 communicating with tubing 4 through which plasma will enter the processing units 40, 60. The conduit 4 has plural positions for stop valves 6 which can occlude the tubing 4 preventing fluids through passage. The tubing 4 communicates through a T fitting 8 to divide plasma into two branches, a first branch 12 which leads to the thrombin processing unit 40 and a second branch 14 leading to the clotting and adhesive proteins processing unit 60. The first valve branch 12 also includes a stop valve 6.

Viewing figure 1B, prior to the introduction of plasma through the first branch 12 thrombin processing unit 40, reagent from preloaded syringe 95 is injected pushing plunger mechanism 94 in the direction of A', into receiving system 20 through sterile barrier filter 92. The reagent passes through one way valve 91; Y connector 90, that merge coupling 18 and valve 91, through branch tubing 93; and finally into the interior of casing 22. Referring to figure 3B and 7B, a valve 24 initially directs the reagent to a reaction chamber 26.

Since it is preferred that the blood product admitted to the inlet 2 be plasma, the whole blood is first processed either by filtering, centrifugation, or another means of settling to remove the heavier red blood cells from the blood products, leaving plasma therebeyond for use in the figure 1 device. Although this system can be dimensioned for any size batch, the plasma required for the thrombin processing unit will typically be 9-10 ml so that the final volume of concentrated thrombin matches a typical yield of cryoprecipitated clotting and adhesive proteins from the clotting and adhesive proteins processing unit 60.

In the embodiments shown in figures 1A and 1B, sealed bags 16 and 78 overlie both the thrombin dispensing syringe 42 (and a lead in of conduit 64) and cryoprecipitate storage tube 76 to provide sterility until both storage containers are introduced into a sterile surgical field (e.g., operatory). Prior to that, the thrombin processing unit 40 operates as shown and described with reference to figures 2A through 10. Viewing figure 1B, after reagent is added, plasma enters the first branch 12 and passes beyond a coupling 18, through tubing branch 93, and into an interior of the casing 22.

Referring back to figure 1A, the thrombin processing unit 40 operates as shown and described with reference to figures 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A and 10. As mentioned, fluid enters the first branch 12 and (figure 1A) passes beyond a coupling 18 and into an interior of a casing 22. Coupling 18 is preferably frictionally and/or adhesively attached to the first branch 12 yet the thrombin processing unit 40 can still be removed (e.g. figure 2A) from the processing set 10 (e.g., by merely detaching or severing branch 12 followed perhaps with heat sealing) after receiving the plasma as shown in figure 2. If adhesive is used, it is a sterile grade for use in an operatory.

Referring to figure 3A, a valve 24 initially directs the plasma to a reaction chamber 26 having an interior tube 28a (figure 6A) preferably formed from glass and capable of receiving a volume, for example 15 ml. Glass tube 28a is preferably shorter than and circumscribed by an overlying barrel 32 preferably formed from PVC. A window 31a in the PVC barrel 32 can be used to gauge and/or verify the contents within the glass tube 28a. Gauging may also include gradations 29, indicating a volume on the glass tube. The glass tube 28a of the reaction chamber 26 receives the plasma from the first branch 12 and into its interior for mixing with reagents preloaded in the glass tube 28a and described hereinafter. As shown in figure 7A, the interior of the glass tube is preferably prefilled only partially with beads 25 preferably formed from borosilicate, glass or ceramic to enhance the reaction and agitation.

Referring to figure 3B, a valve 24 initially directs the plasma to a reaction chamber 26 having tube 28b (figure 6B) preferably formed from clear polycarbonate and capable of receiving a volume, for example, 15 ml. Graduated lines 31b on the polycarbonate tube 28b can be used to gauge the contents within the tube 28b. The

polycarbonate tube 28b of the reaction chamber 26 receives the plasma from the first branch 12 and into the interior for mixing with reagents previously added into the polycarbonate tube 28b and described hereinafter. As shown in figure 7B, the interior of the tube 28b is preferably prefilled only partially with beads 25 preferably
5 formed from borosilicate or ceramic to enhance the reaction and agitation.

The reaction chamber 26 of the embodiment shown in figures 1A and 3A is formed with first and second end caps 34 detailed in figures 6A, 7A and 8A. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite
10 end. Each spout 36 is isolated from the beads 25 by a screen 23 nested within necked-down portion 48. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each
15 valve and its spout into the interior of the glass tube 28a and through flow is controlled by the valves 24, 44. As shown in figure 8A, the cap 34 includes an annular necked-down portion 48 which frictionally and/or adhesively resides within an interior hollow of the PVC barrel 32. In this way, the necked-down portion 48 rests upon ends of the glass tube 28a in sealing engagement therewith,
20 isolating the interior of the reaction chamber from the PVC barrel 32.

For the embodiment forming the reaction chamber 26 of the embodiment shown in figures 1B and 3B mainly out of polycarbonate tube 28 is detailed in figures 6B, 7B and 8B. This reaction chamber 26 is formed with first and second end caps 34 detailed in figure 8B. Each end cap includes a central outwardly conically
25 tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 has interior obstructions preventing passage of beads 25 while allowing passage of fluid. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a
30 respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the polycarbonate tube 28b and through flow is controlled by the valves 24, 44. As

shown in figure 8B, the cap 34 includes an annular interior recess portion 48 which adhesively resides on the interior surface of the polycarbonate tube 28b.

Preferably, ethanol and calcium chloride are the reagents which have been preloaded into the reaction chamber 26 or within reagent syringe 95. Initially, both valves 24 and 44 are oriented so that reagents will not pass therebeyond to seal the chamber for the embodiment of figure 1A. Viewing figure 1B, initially valve 24 is oriented so plasma will not enter reaction chamber 26, and valve 44 is oriented to allow passageway between the reaction chamber 26 and the draw plunger 56. Referring back to figure 1A, after the plasma has been pumped into processing unit 60, valve 44 is turned to allow access to the draw plunger 56 and valve 24 is oriented to allow access between the passageway 21 and the reaction chamber 26. Slide clip 6 is opened with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1A, syringe 56 plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. Referring back to figure 1B, the reagent syringe 95 is attached to open end of sterile barrier filter 92. Plunger 94 is depressed to transfer reagent syringe through sterile barrier filter and passageway 93 to reaction chamber 26. Likewise to the figure 1A embodiment, the figure 1B, with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1B, the syringe plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. In both embodiments syringe 56 includes a filter 62 located in the flow path. More specifically, the path 43 between valve 44 and syringe 56 includes a filter 62 located in the flow path. The filter 62 provides an aseptic microbial barrier so that, upon subsequent delivery of the thrombin to the dispensing syringe 42 (figure 1), there is no contamination from around the seal 57 of plunger 58 delivered to syringe 42. Plasma will subsequently enter chamber 26 from conduit 4 to replace air. Valve 24 is oriented to address filter 66. The reagents and plasma are briefly agitated assisted by beads 25 (and allowed to incubate for about 40 to 70 minutes). After incubation, thrombin processing unit 40 is agitated to loosen and break up gel formation. For the embodiment of figure 1B, the thrombin processing unit 40 is then returned to a motionless horizontal position for no less than 10 minutes. Afterwards the thrombin processing unit 40 is again agitated to loosen and break up gel formation. For both embodiments, the plunger of syringe 56 is pushed in the direction opposite arrow A to move thrombin from chamber 26

through filter 66 into syringe 42. Delivery of thrombin to syringe 42 can be enhanced by retracting plunger 43 of syringe 42, defining a push pull system. Filter 66 removes particulate matter from the thrombin, including gel.

5 By allowing the thrombin contained in the reaction chamber 26 to reside therein after agitation for no less than 10 minutes enhances the effectiveness of the filter 66 in removing particulate matter for subsequent utilization. The time span for conversion and activation allows enough particulate matter to be removed by the filter to optimize the use of the thrombin later in a narrow orificed dispenser, such as a sprayer, or expression through a thin tube.

10 Figures 9A, 9B and 10 reveal alternative embodiments of filter 66 which includes an outer cylindrical wall 65 with end caps 34 each having a cylindrical spout 37 circumscribed by an annular recess 39. The alternative embodiment shown in figure 9A shows the centrally disposed cylindrical filter element 67a is preferably formed from polyurethane foam. While as shown in figure 9B the centrally
15 disposed cylindrical filter element 67b is preferably formed from rolled polyester. Also shown in figure 9B, are circular filters 68 preferably formed from glass fiber or polyester. In each alternative embodiment, filter 67a or 67b filters by weight, size and protein binding.

Referring back to figures 1A and 1B, attention is now directed to the clotting
20 and adhesive protein processing unit 60. All of the plasma not diverted to the thrombin processing unit 40 is admitted to an interior chamber 72 of the clotting and adhesive protein processing unit 60. The clotting and adhesive protein processing unit 60 is manipulated by heat exchange and rotation so that all clotting and adhesive proteins extracted from the plasma will sediment at a nose 74 of the
25 chamber 72 for subsequent extraction by means of a clotting and adhesive protein collection tube or dispensing syringe 76 contained in a sterile pouch 78. Chamber 72 is protected during this process by a filter vent 82 preventing contamination. Once the thrombin has been loaded into the dispensing syringe 42, and the clotting and adhesive proteins have been loaded into the clotting and adhesive collection tube or
30 dispensing syringe 76, the two storage containers 42, 76 can be decoupled from the processing set 10 (e.g. sterile disconnect device), and passed near the sterile, surgical arena. The overwrap bags are subsequently opened, and the storage containers 42, 76 are decoupled and transferred into the surgical area where the contents are

dispensed into individual sterile 3cc plastic syringes which are subsequently loaded into the fibrin glue applicator for spraying or line and dot application. Mixing the thrombin with the clotting and adhesive proteins forms the biological glue.

Both dispensing syringes 42 and 76 are stored at room temperature, or preferably stored at their optimal conditions: cryoprecipitate 76 being stored at room temperature and thrombin 42, stored in an ice bath at 1°C to 5°C. Please see figures 13 through 16.

Assume 9-10 ml of room temperature plasma is introduced into the reaction chamber 26. Other plasma volumes have utility. Please see figures 15 and 16. Add 1.0 ml of 75 mM calcium chloride (CaCl_2) and 2.0 ml of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and added to comprise 18.9% volume/unit volume or 15.02% ethanol weight/unit volume). Other ratios of reagent volume, comprising of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and a stock solution of 75 mM calcium chloride (CaCl_2)), to plasma volume have utility phase. Please see figures 13 and 14. The thrombin life span is shown to have been at least 300 minutes while its clotting time is at 2.98 seconds. An ethanol final concentration range between 8.0% and 20.0% (volume/unit volume), however, still has utility. Please see figure 11.

When the ethanol is at a final concentration of 18.9% volume/unit volume (as above) and the calcium chloride final concentration is 5.7 mM (1 ml taken from a 75 mM stock solution of calcium chloride), the thrombin lifespan also extends to at least 360 minutes while maintaining a clot time of 5.98 seconds when thrombin is stored at room temperature. Storing thrombin in optimal 1°C to 5°C ice bath typically maintains lot times of 2 to 3 seconds at 360 minutes. Calcium chloride stock solution concentrations ranging between 50 mM and 250 mM, however, have utility. Please see figure 12. The final concentrations range from 4.5mM to 23 mM.

Solutions such as saline, dilute CaCl_2 (e.g. 40mM to 125 mM CaCl_2) or even sterile water added to the thrombin can alter both the clotting time and life span of the thrombin. Assume an ethanol final concentration of 18.9% and a final calcium chloride concentration of 5.7 mM was used in the reaction chamber 26. When the thrombin has been diluted 1 to 1.5 with water, the clot time has been extended to just less than 30 seconds, and has a life span of up to 150 minutes.

Moreover, having thus described the invention, it should be apparent that numerous structural modifications and adaptations may be resorted to without departing from the scope and fair meaning of the instant invention as set forth hereinabove and as described hereinbelow by the claims.

Claims

We Claim:

Claim 1 - A method for generating autologous thrombin from a patient, the steps including:

- 5 obtaining a blood product from the patient;
 sequestering plasma from the product;
 enriching the prothrombin in a plasma fraction;
 converting the prothrombin to thrombin, and
 filtering particulate from the thrombin.

10 Claim 2 - The method of claim 1 further including the step of altering the clotting time.

 Claim 3 - The method of claim 2 including adding ethanol to enrich the prothrombin in a plasma fraction.

15 Claim 4 - The method of claim 3 wherein the converting step includes adding CaCl_2 .

 Claim 5 - The method of claim 4 including centrifuging the blood product for obtaining plasma.

20 Claim 6 - The method of claim 2 wherein a predictable clotting time extension occurs through diluting the thrombin with any of the group consisting of saline, CaCl_2 solution and sterile water.

 Claim 7 - The method of claim 6 including filtering the plasma by weight, size and protein binding.

 Claim 8 - A method for producing fast clotting autologous thrombin which is stable for more than fifteen minutes, the steps including:

25 sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

 Claim 9 - Autologous thrombin which provides fast clotting in less than five seconds for more than fifteen minutes.

Claim 10 - A composition for extracting thrombin from plasma consisting essentially of:

Plasma;

Ethanol (ETOH);

5 CaCL₂.

Claim 11 - The composition of claim 10 wherein ETOH is present at 18.9% and CaCL₂ is present at 23.0 mM both by volume in final concentration.

Claim 12 - The composition of claim 10 wherein ETOH is present at 18.9% and CaCL₂ is present at 5.7 mM both by volume in final concentration.

10 Claim 13 - The composition of claim 10 wherein ETOH is present at a range between 8% and 20% and CaCL₂ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 14 - A method for preparing thrombin comprising:

obtaining plasma;

15 adding ETOH and CaCL₂ to the plasma, forming a composition:

agitating the composition;

filtering the composition of particulate, thereby passing the thrombin through the filter.

20 Claim 15 - The method of claim 14 whereby subsequent to agitating the composition, incubating the composition for an amount of time greater than or equal to ten minutes.

Claim 16 - The method of claim 15 whereby prior to filtering the composition, re-agitating the composition.

25 Claim 17 - A device for preparing thrombin from plasma, comprising:
a reaction chamber having a solution of CaCL₂ and ETOH therein;
means for admitting plasma into said reaction chamber;
a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 18 - An autologous biological glue processing device, comprising, in combination:

- 5 a thrombin processing means,
 a clotting and adhesive proteins processing means operatively coupled to said thrombin processing means,
 means for receiving plasma via said operative coupling for subsequent conversion of the plasma to, respectively thrombin and clotting and adhesive
10 proteins.

Claim 19 - A device for preparing thrombin from plasma, comprising:

- a reaction chamber having ceramic beads or borosilicate glass therein;
 means for admitting a reagent into said reaction chamber;
 means for admitting plasma into said reaction chamber;
15 a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and
 a filter located between said reaction chamber and said thrombin receiving syringe.

- Claim 20 - The device of claim 19 wherein the reagent includes CaCl_2 and
20 ETOH solution.

Claim 21 - The method of claim 1 further including the step of contacting the plasma with glass beads.

Claim 22 - A composition for extracting thrombin from plasma consisting essentially of:

- 25 plasma;
 ethanol (ETOH);
 CaCl_2 ; and
 glass beads.

Claim 23 - The composition of claim 22 wherein ETOH is present at 18.9% and CaCl_2 is present at 23.0 mM both by volume in final concentration.

Claim 24 - The composition of claim 22 wherein ETOH is present at 18.9% and CaCl_2 is present at 5.7 mM both by volume in final concentration.

5 Claim 25 - The composition of claim 22 wherein ETOH is present at a range between 8% and 20% and CaCl_2 is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 26 - An apparatus to prepare thrombin from plasma consisting of:
a reacting chamber to accept CaCl_2 and ethanol means include
10 injection of plasma into said reacting chamber;
a syringe to receive thrombin connected to said reacting chamber;
and a filter between said reacting chamber and syringe which is to
receive thrombin.

15 Claim 27 - The apparatus of claim 26 further including glass beads beforehand.

AMENDED CLAIMS

[received by the International Bureau on 27 October 2000 (27.10.00);
original claims 1,4-6, 8-14, 17, 18, 20 and 22-27 amended; new claims 28-53 added; other
claims unchanged (7 pages)]

Claim 1 - A method for generating autologous thrombin from a patient, the steps including:

- 5 obtaining a blood product from the patient;
 sequestering unadulterated plasma from the product;
 enriching the prothrombin in an unadulterated plasma fraction;
 converting the prothrombin to thrombin, and
 filtering particulate from the thrombin.

10 Claim 2 - The method of claim 1 further including the step of altering the clotting time.

 Claim 3 - The method of claim 2 including adding ethanol to enrich the prothrombin in a plasma fraction.

15 Claim 4 - The method of claim 3 wherein the converting step includes adding a source of calcium ions.

 Claim 5 - The method of claim 4 including centrifuging the blood product for obtaining unadulterated plasma.

20 Claim 6 - The method of claim 2 wherein a predictable clotting time extension occurs through diluting the thrombin with any of the group consisting of saline, CaCl₂ solution and sterile water.

 Claim 7 - The method of claim 6 including filtering the plasma by weight, size and protein binding.

25 Claim 8 - A method for producing fast clotting autologous thrombin which is stable for more than fifteen minutes, the steps including:
 sequestering prothrombin from unadulterated plasma and converting the prothrombin to thrombin.

 Claim 9 - Autologous thrombin which provides fast clotting in less than five seconds and is stable for more than fifteen minutes.

Claim 10 - A composition for extracting thrombin from plasma consisting essentially of:

unadulterated Plasma;

Ethanol (ETOH);

5 CaCl₂.

Claim 11 - The composition of claim 10 wherein ETOH is present at 18.9% and CaCl₂ is present at 23.0 mM both by volume in final concentration.

Claim 12 - The composition of claim 10 wherein ETOH is present at 18.9% and CaCl₂ is present at 5.7 mM both by volume in final concentration.

10 Claim 13 - The composition of claim 10 wherein ETOH is present at a range between 8% and 20% and CaCl₂ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 14 - A method for preparing thrombin comprising:

obtaining unadulterated plasma;

15 adding ETOH and CaCl₂ to the unadulterated plasma, forming a composition:

agitating the composition;

filtering the composition of particulate, thereby passing the thrombin through the filter.

20 Claim 15 - The method of claim 14 whereby subsequent to agitating the composition, incubating the composition for an amount of time greater than or equal to ten minutes.

Claim 16 - The method of claim 15 whereby prior to filtering the composition, re-agitating the composition.

25 Claim 17 - A device for preparing thrombin from plasma, comprising:
a reaction chamber having a solution of CaCl₂ and ETOH therein;
means for admitting unadulterated plasma into said reaction chamber;
a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 18 - A single donor biological glue processing device, comprising, in combination:

- 5 a thrombin processing means,
 a clotting and adhesive proteins processing means operatively coupled to said thrombin processing means,
 means for receiving plasma via said operative coupling for subsequent conversion of the plasma to, respectively thrombin in said thrombin processing
10 means and clotting and adhesive proteins in said clotting and adhesive proteins processing means.

Claim 19 - A device for preparing thrombin from plasma, comprising:

- a reaction chamber having ceramic beads or borosilicate glass therein;
 means for admitting a reagent into said reaction chamber;
15 means for admitting plasma into said reaction chamber;
 a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and
 a filter located between said reaction chamber and said thrombin receiving syringe.

- 20 Claim 20 - The device of claim 19 wherein the reagent includes CaCl_2 and ETOH solution.

Claim 21 - The method of claim 1 further including the step of contacting the plasma with glass beads.

- 25 Claim 22 - A composition for extracting thrombin from plasma consisting essentially of:

 plasma;
 ethanol (ETOH);
 CaCl_2 ; and
 glass beads.

Claim 23 - The composition of claim 22 wherein ETOH is present at 18.9% and CaCl₂ is present at 23.0 mM both by volume in final concentration.

Claim 24 - The composition of claim 22 wherein ETOH is present at 18.9% and CaCl₂ is present at 5.7 mM both by volume in final concentration.

5 Claim 25 - The composition of claim 22 wherein ETOH is present at a range between 8% and 20% and CaCl₂ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 26 - An apparatus to prepare thrombin from plasma consisting of:
a reacting chamber to accept CaCl₂ and ethanol, and means for delivery
10 of plasma into said reacting chamber;
a syringe connected to said reacting chamber to receive thrombin from said reacting chamber;
and a filter between said reacting chamber and syringe which is to receive thrombin.

15 Claim 27 - The apparatus of claim 26 further including glass beads in said reacting chamber.

Claim 28 - A method for generating and then dispensing thrombin, the steps including:

20 taking whole blood from one person,
sequestering prothrombin from the whole blood,
converting the prothrombin to thrombin,
loading the thrombin into a syringe,
and then applying the thrombin onto an area to stem blood flow.

Claim 29 - The method of claim 28 including loading clotting proteins into
25 another syringe and dispensing the clotting proteins concurrently with the thrombin.

Claim 30 - A method for generating thrombin from one person, the steps including:

sequestering prothrombin from plasma taken from the person,

converting the prothrombin to thrombin and removing particulate material therefrom.

Claim 31 - The method of claim 30 further including diluting the thrombin in order to alter clotting time of the thrombin.

5 Claim 32 - The method of claim 31 including adding a source of calcium ions to alter the clotting time.

Claim 33 - The method of claim 32 including adding CaCl_2 to alter the clotting time.

10 Claim 34 - The method of claim 31 including adding saline to alter the clotting time.

Claim 35 - The method of claim 31 including adding sterile water to alter the clotting time.

Claim 36 - The method of claim 2 wherein the step of altering the clotting time includes adding a source of calcium ions.

15 Claim 37 - The method of claim 2 wherein the step of altering the clotting time includes adding CaCl_2 .

Claim 38 - The method of claim 2 wherein the step of altering the clotting time includes adding saline.

20 Claim 39 - The method of claim 2 wherein the step of altering the clotting time includes adding sterile water.

Claim 40 - A method for generating thrombin from one person, the steps including:

25 taking whole blood from the one person,
obtaining plasma from the whole blood,
converting prothrombin in the plasma to thrombin and
sequestering the thrombin from the plasma.

Claim 41 - The method of claim 40 including altering the clotting time of the thrombin to clot in less than five seconds.

Claim 42 - The method of claim ²⁸41 wherein the step of altering the clotting time includes adding a source of calcium ions.

Claim 43 - The method of claim 42 wherein the step of altering the clotting time includes adding CaCl_2 .

5 Claim 44 - The method of claim 41 wherein the step of altering the clotting time includes adding saline.

Claim 45 - The method of claim 41 wherein the step of altering the clotting time includes adding sterile water.

10 Claim 46 - The method of claim 40 including making the thrombin stable for more than fifteen minutes.

Claim 47 - The method of claim 46 including adding a source of calcium ions to alter the clotting time.

Claim 48 - The method of claim 47 including adding CaCl_2 to alter the clotting time.

15 Claim 49 - The method of claim 46 including adding saline to alter the clotting time.

Claim 50 - The method of claim 46 including adding sterile water to alter the clotting time.

20 Claim 51 - The method of claim 1, the steps including adding the reagents ETOH and CaCl_2 to the plasma followed by agitation to form the thrombin.

Claim 52 - The method of claim 51 further including:
taking whole blood from one person,
sequestering prothrombin from the whole blood,
converting the prothrombin to thrombin,
25 loading the thrombin into a syringe,
and then applying the thrombin onto an area to stem blood flow.

Claim 53 - The device of claim 18 including a thrombin syringe coupled to said thrombin processing means to receive thrombin therefrom, said thrombin syringe initially ensconced in a bag, and

a clotting and adhesive protein syringe coupled to said clotting and adhesive protein processing means to receive clotting and adhesive proteins therefrom, said clotting and adhesive protein syringe initially ensconced in a bag.

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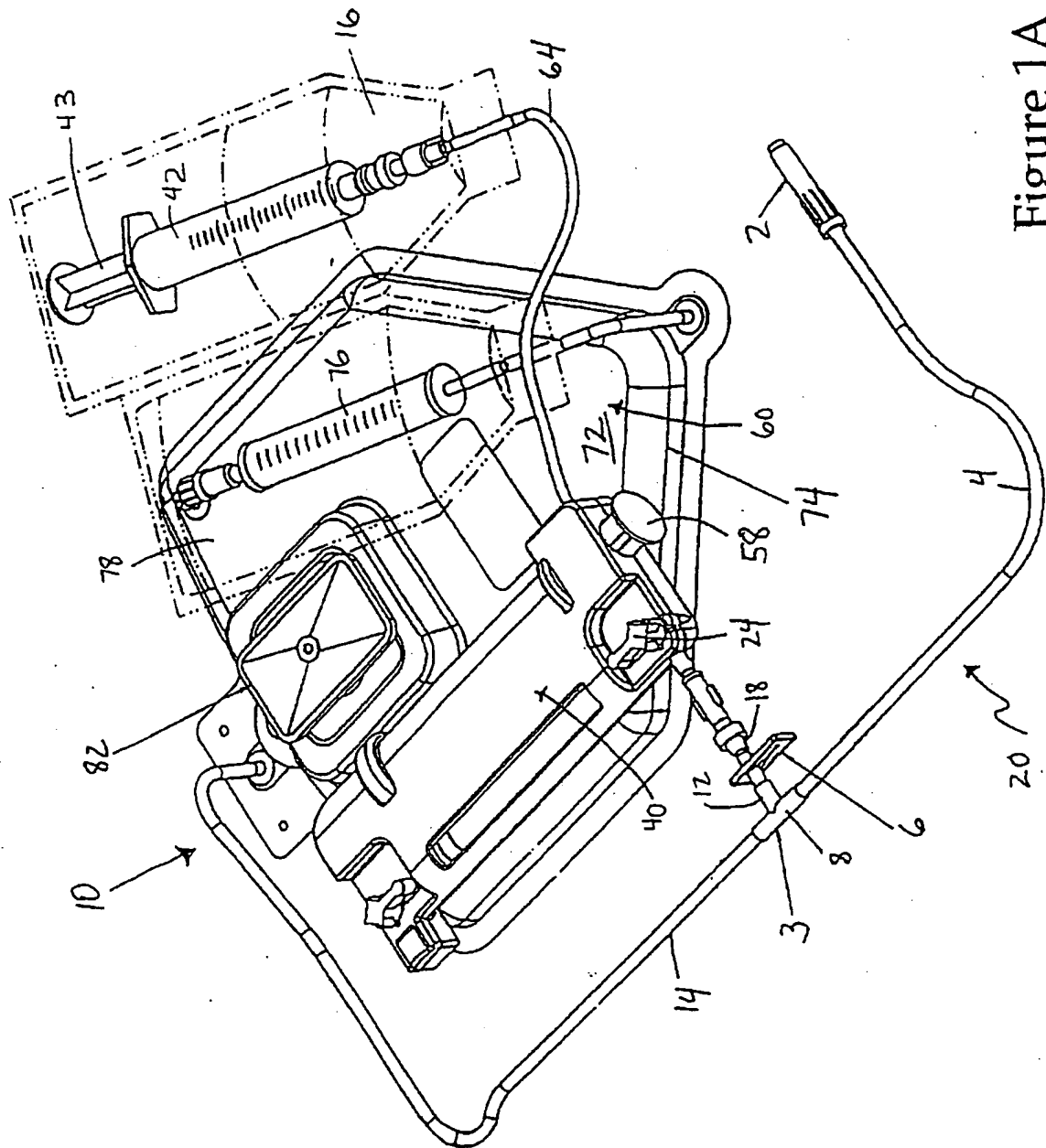
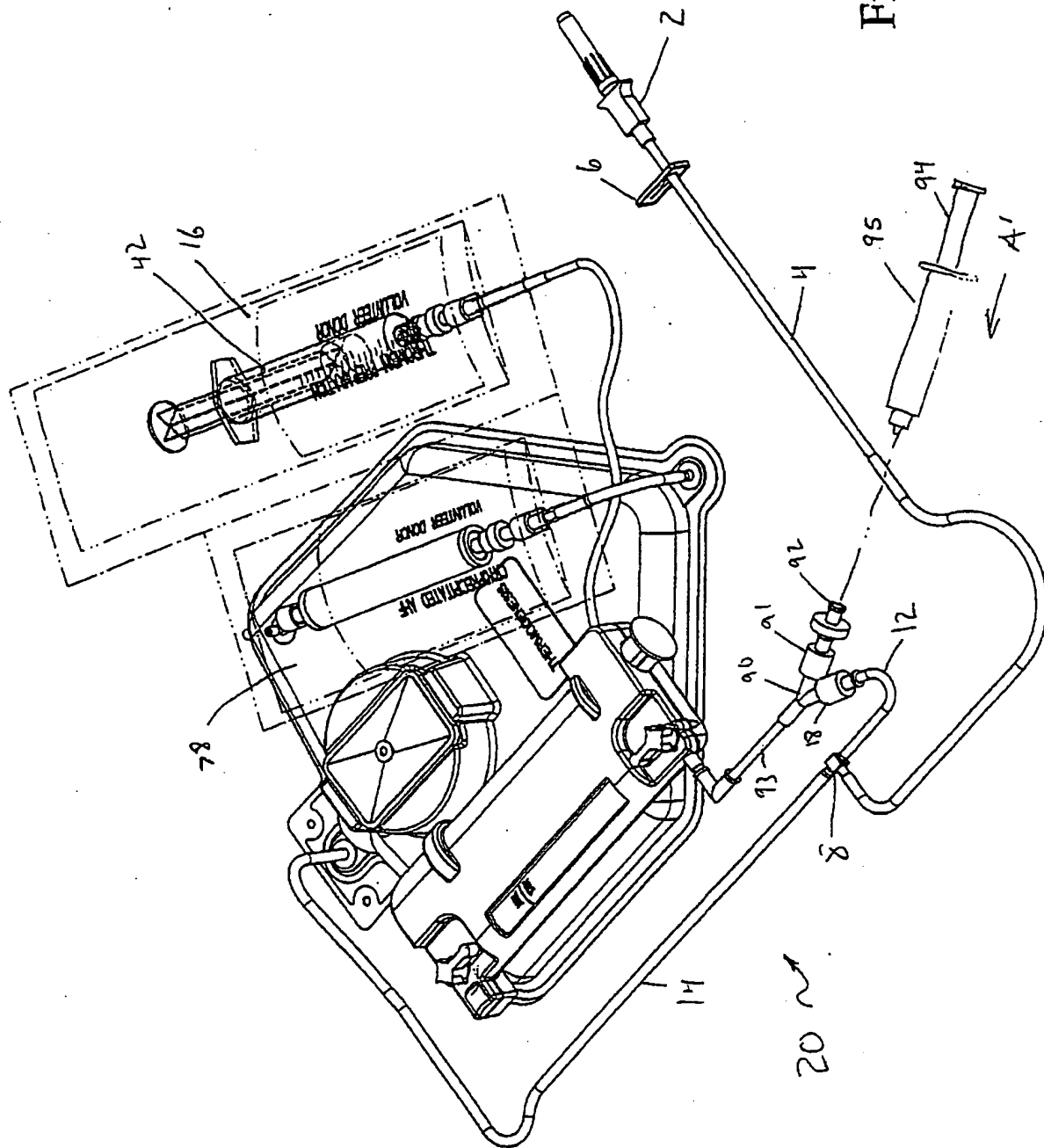


Figure 1A

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Figure 1B



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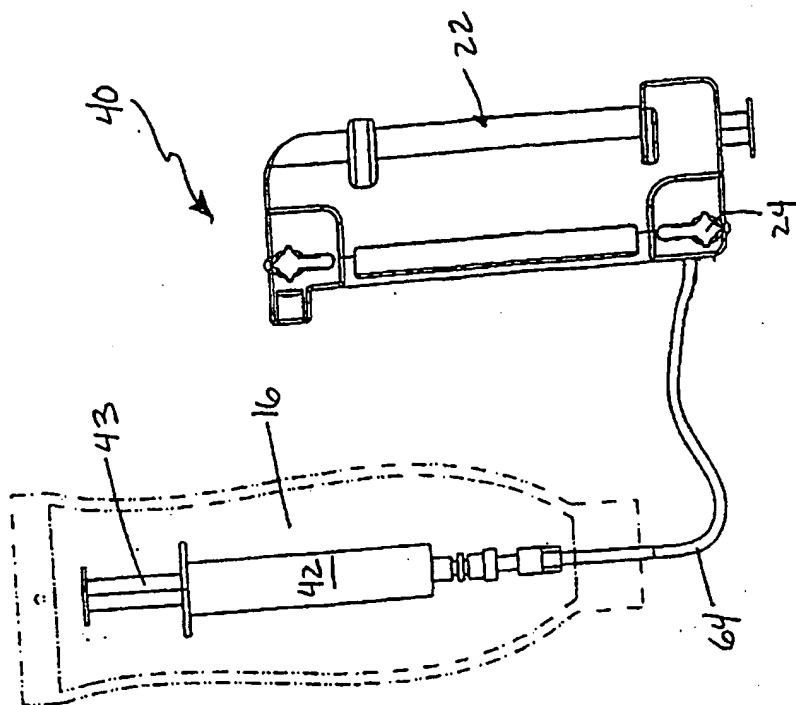


Figure 2B

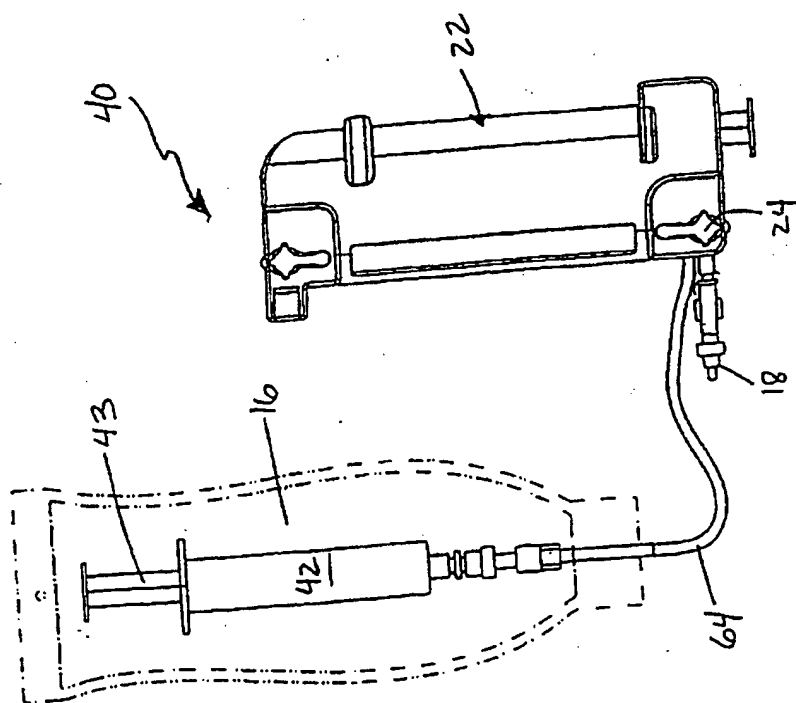


Figure 2A

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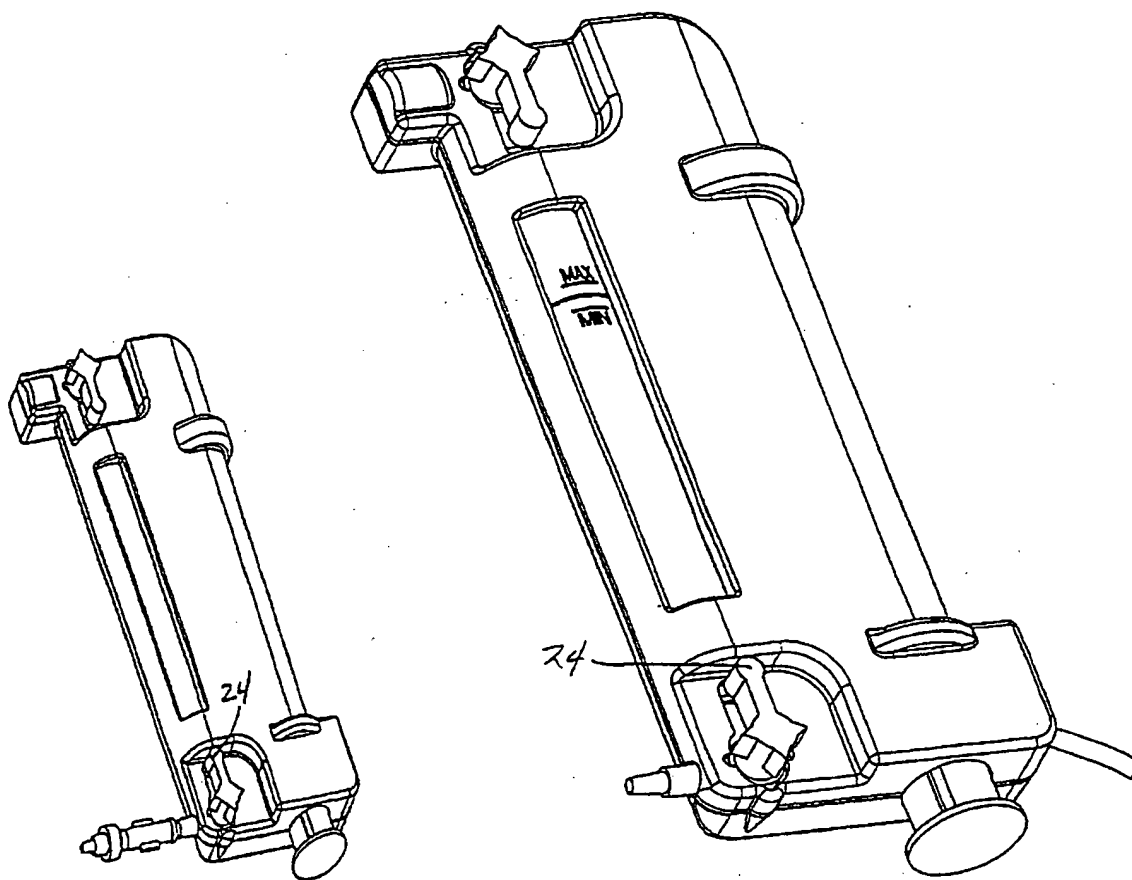


Figure 4A

Figure 4B

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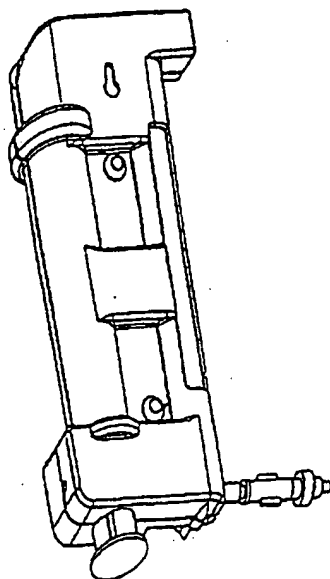


Figure 5A

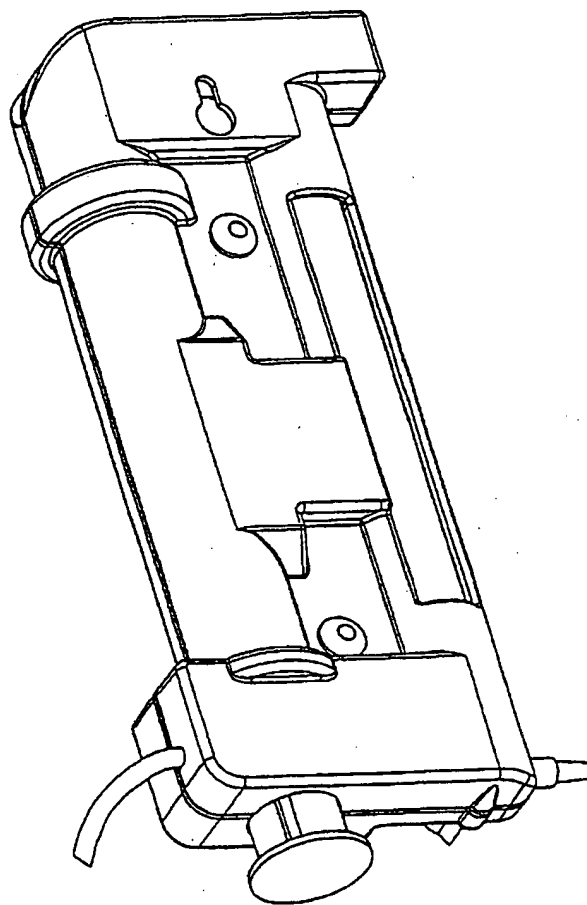


Figure 5B

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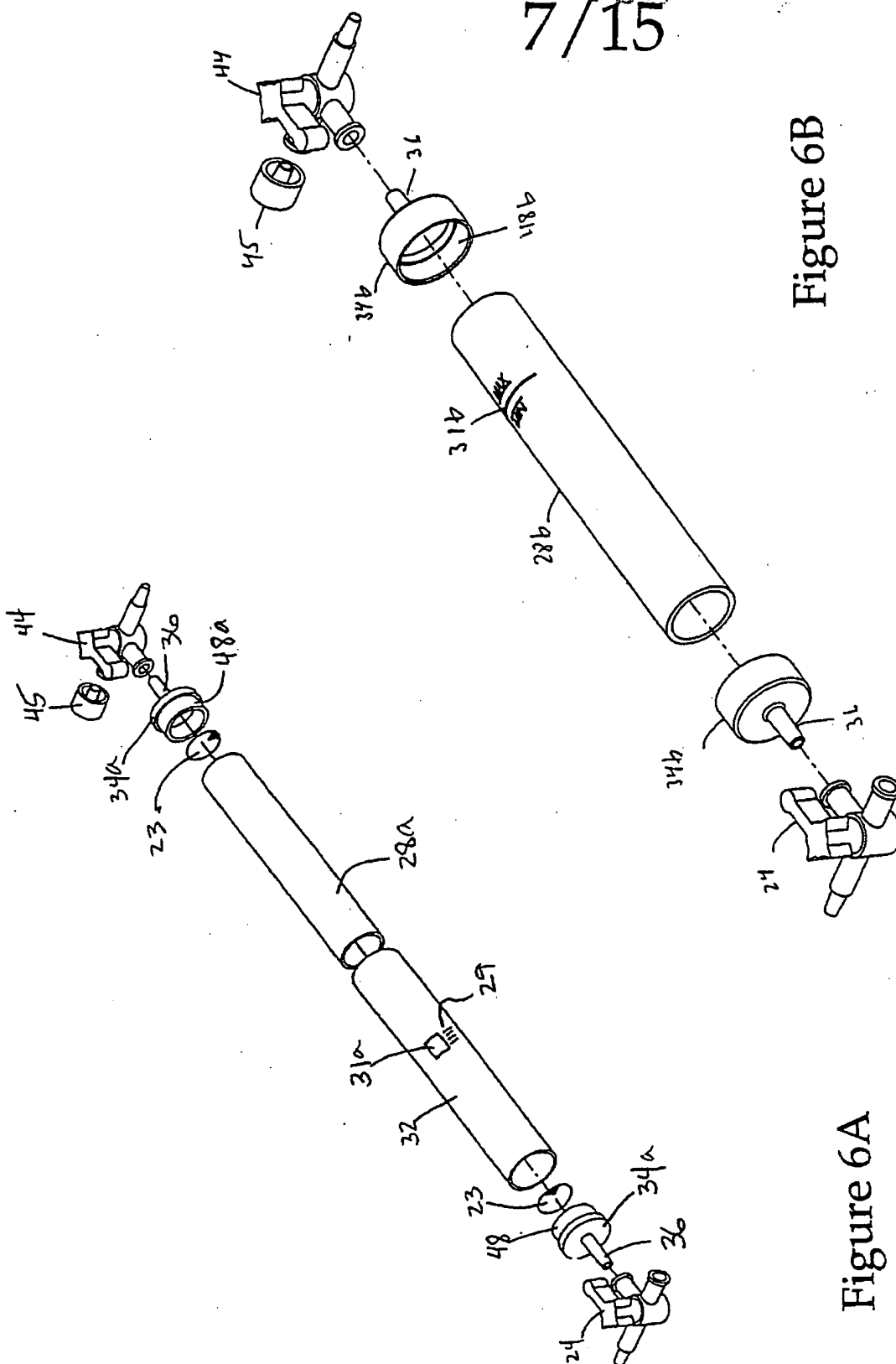


Figure 6B

Figure 6A

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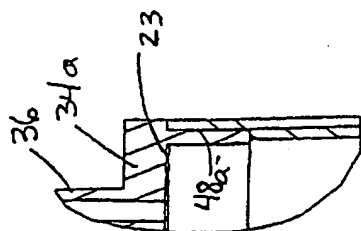


Figure 8A

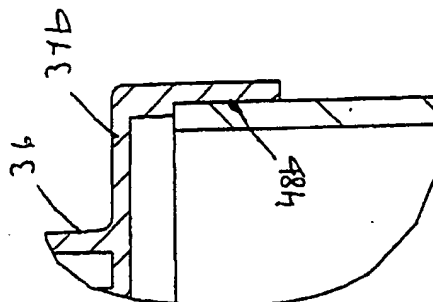


Figure 8B

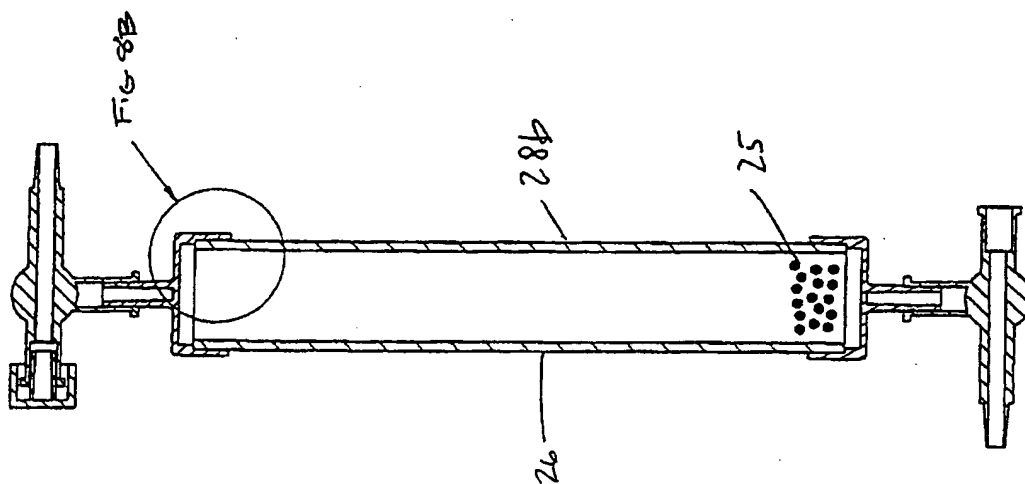


Figure 7B

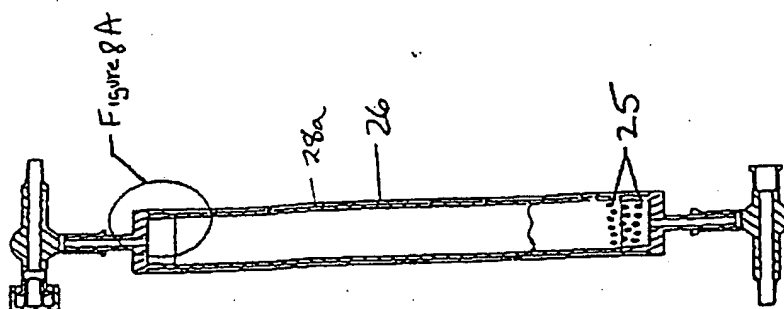


Figure 7A

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Figure 10

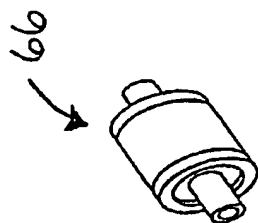


Figure 9B

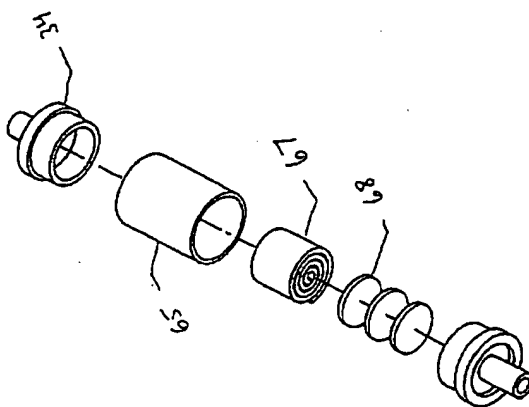
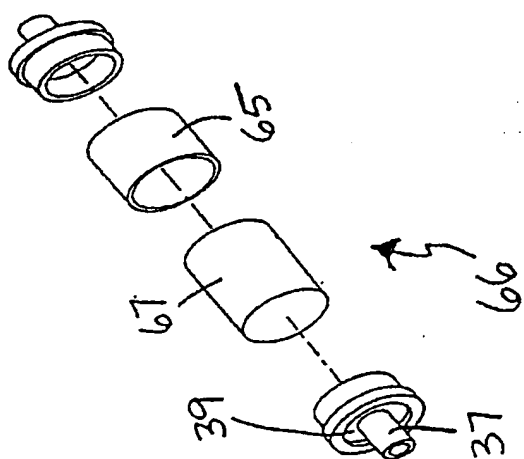


Figure 9A



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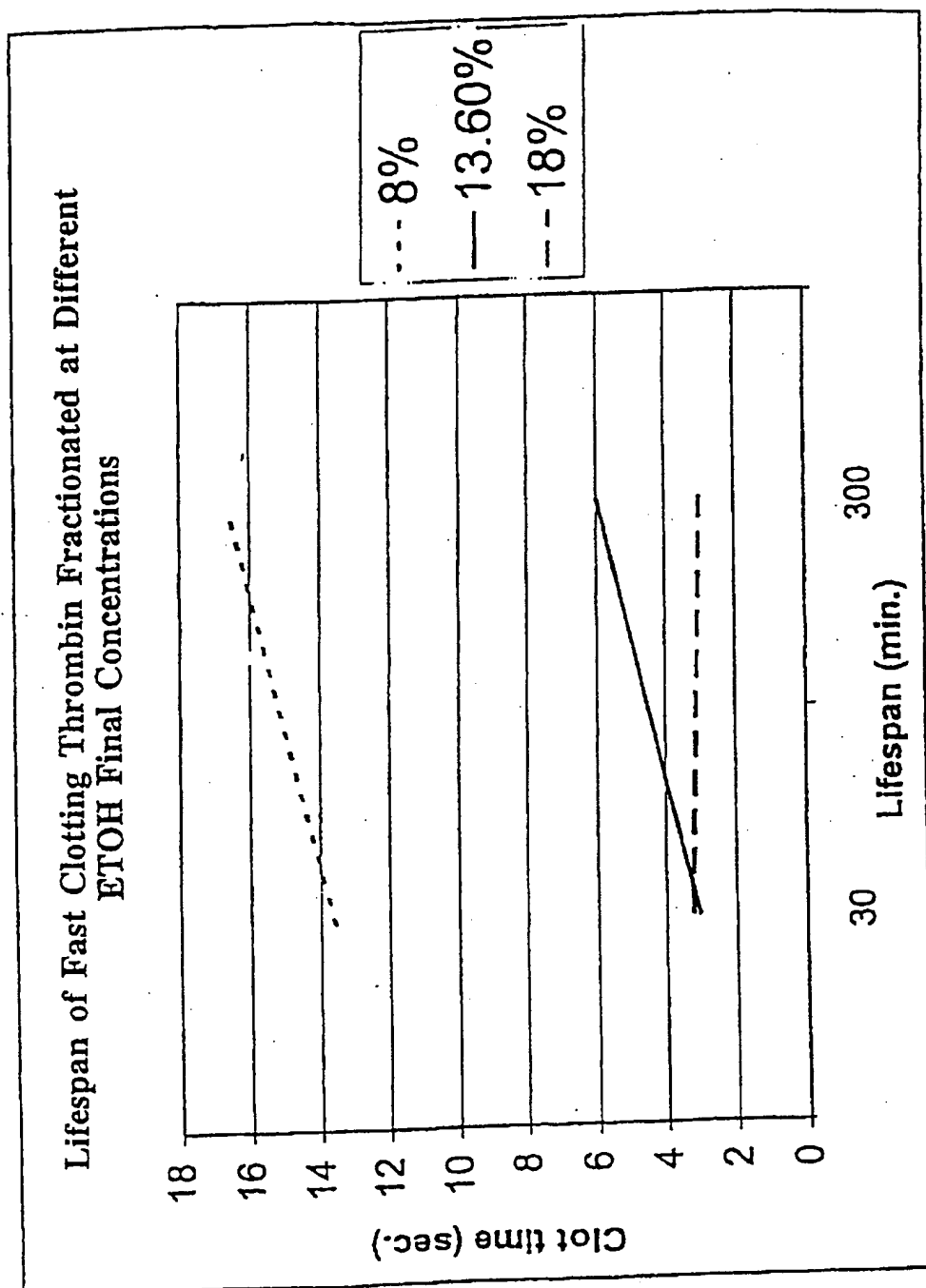


Figure 11

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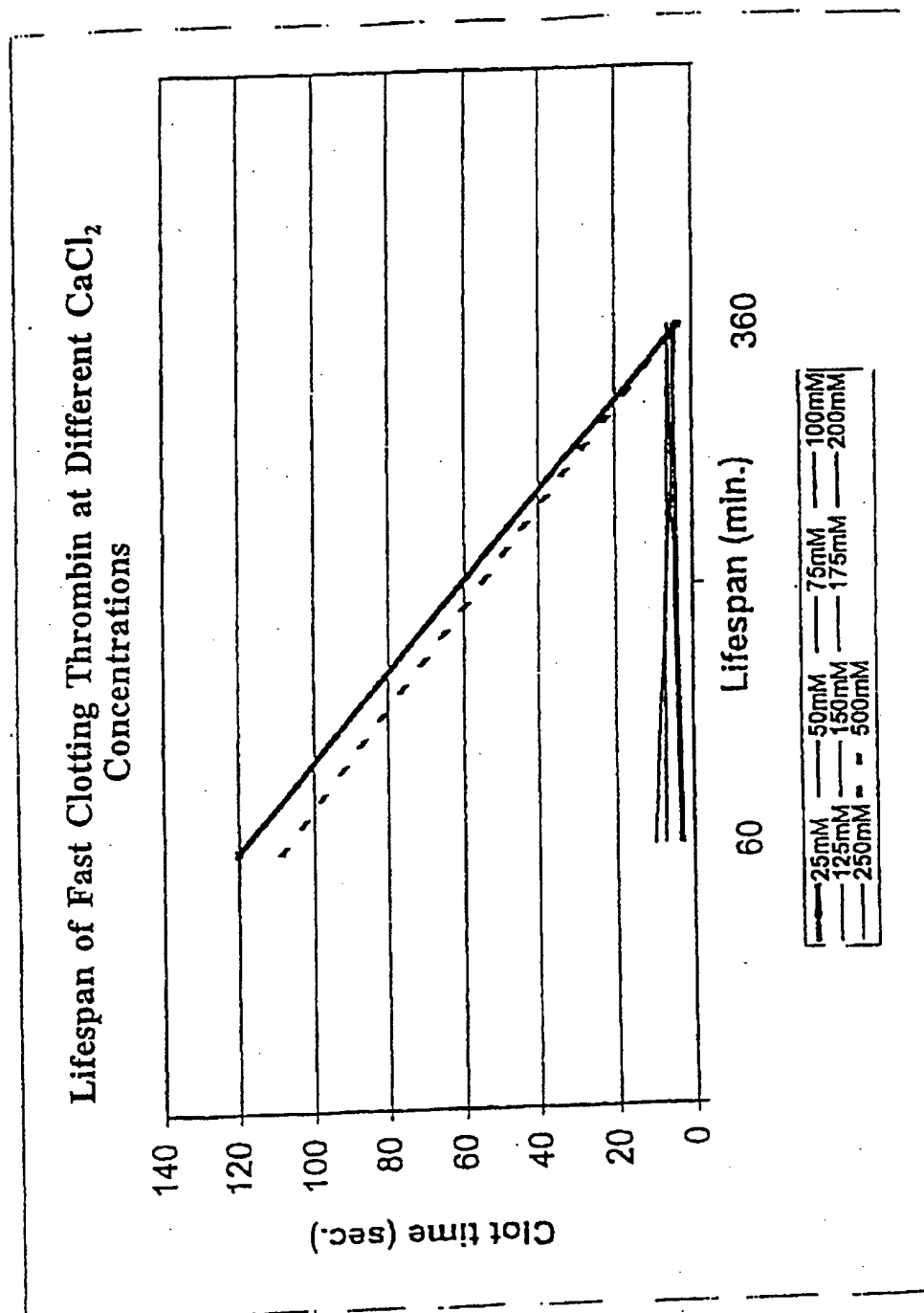


Figure 12

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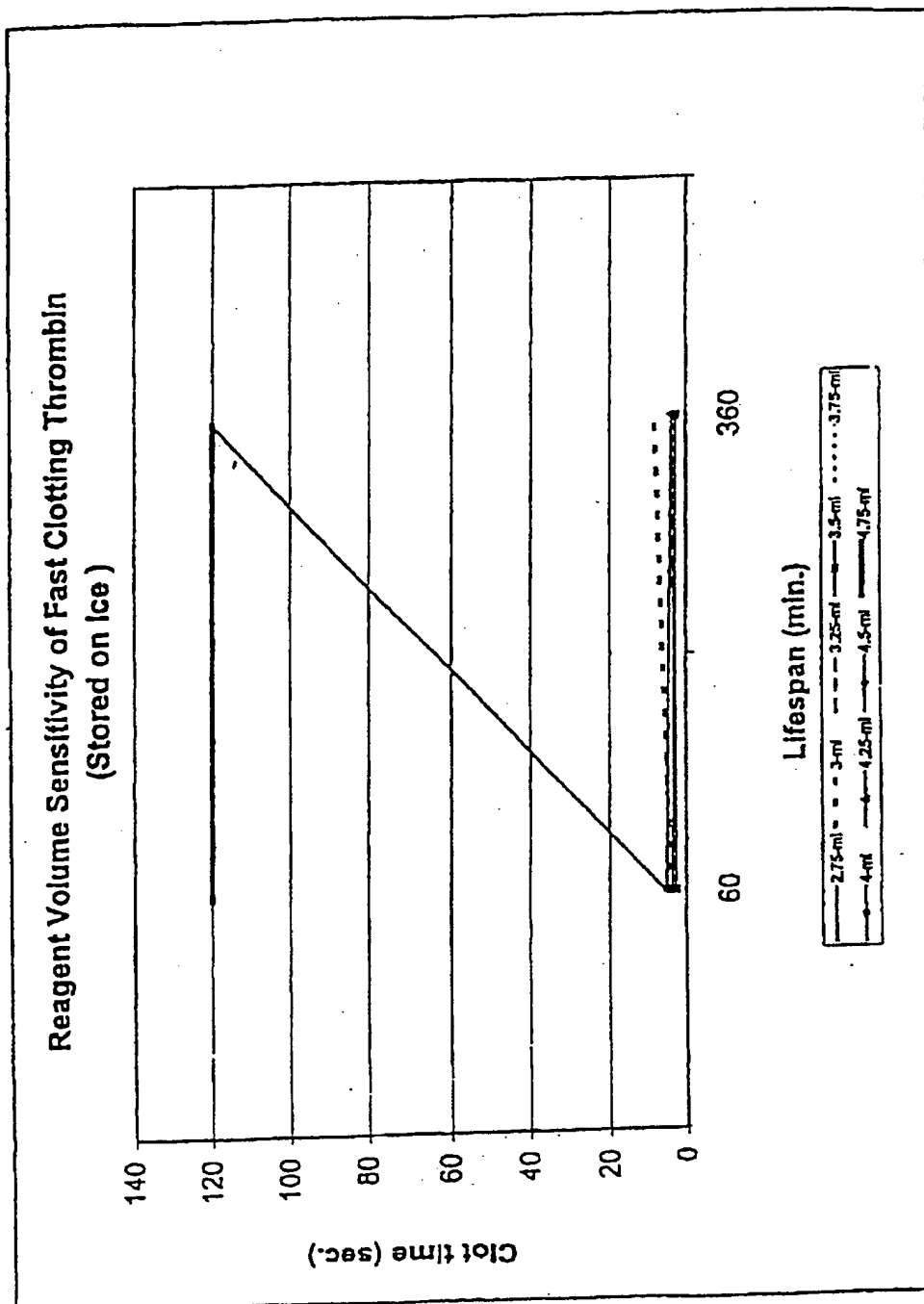


Figure 13

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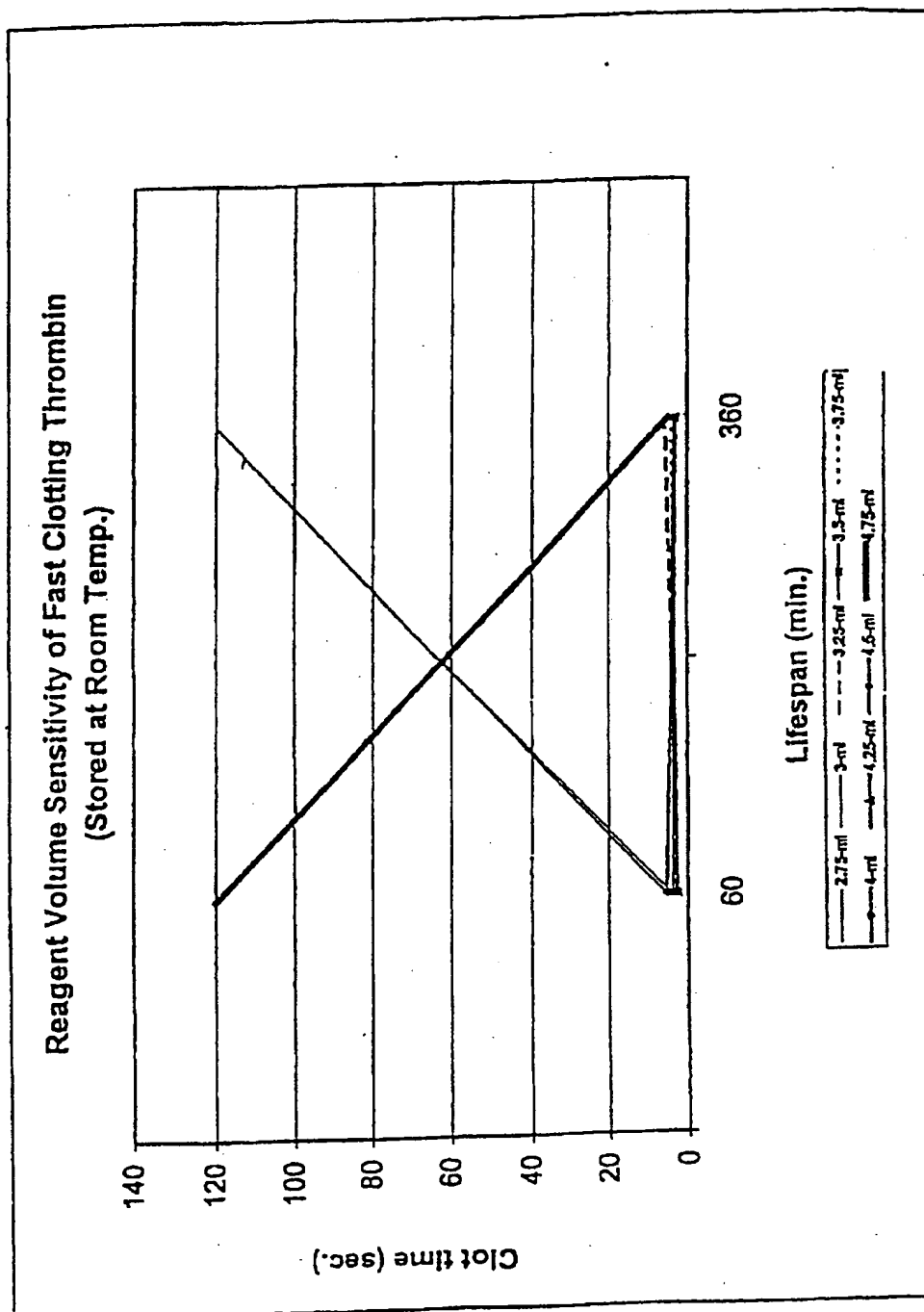


Figure 14

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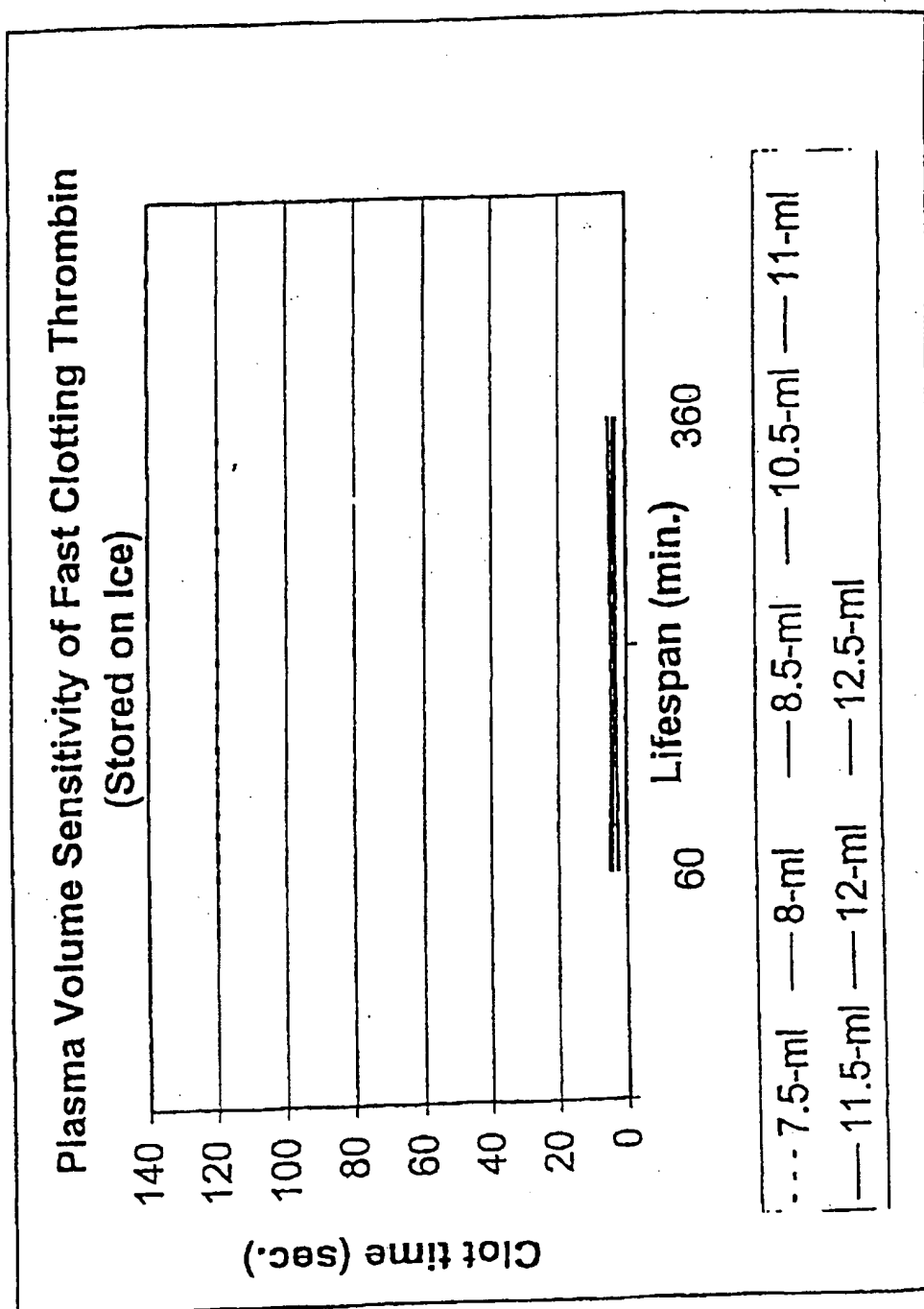


Figure 15

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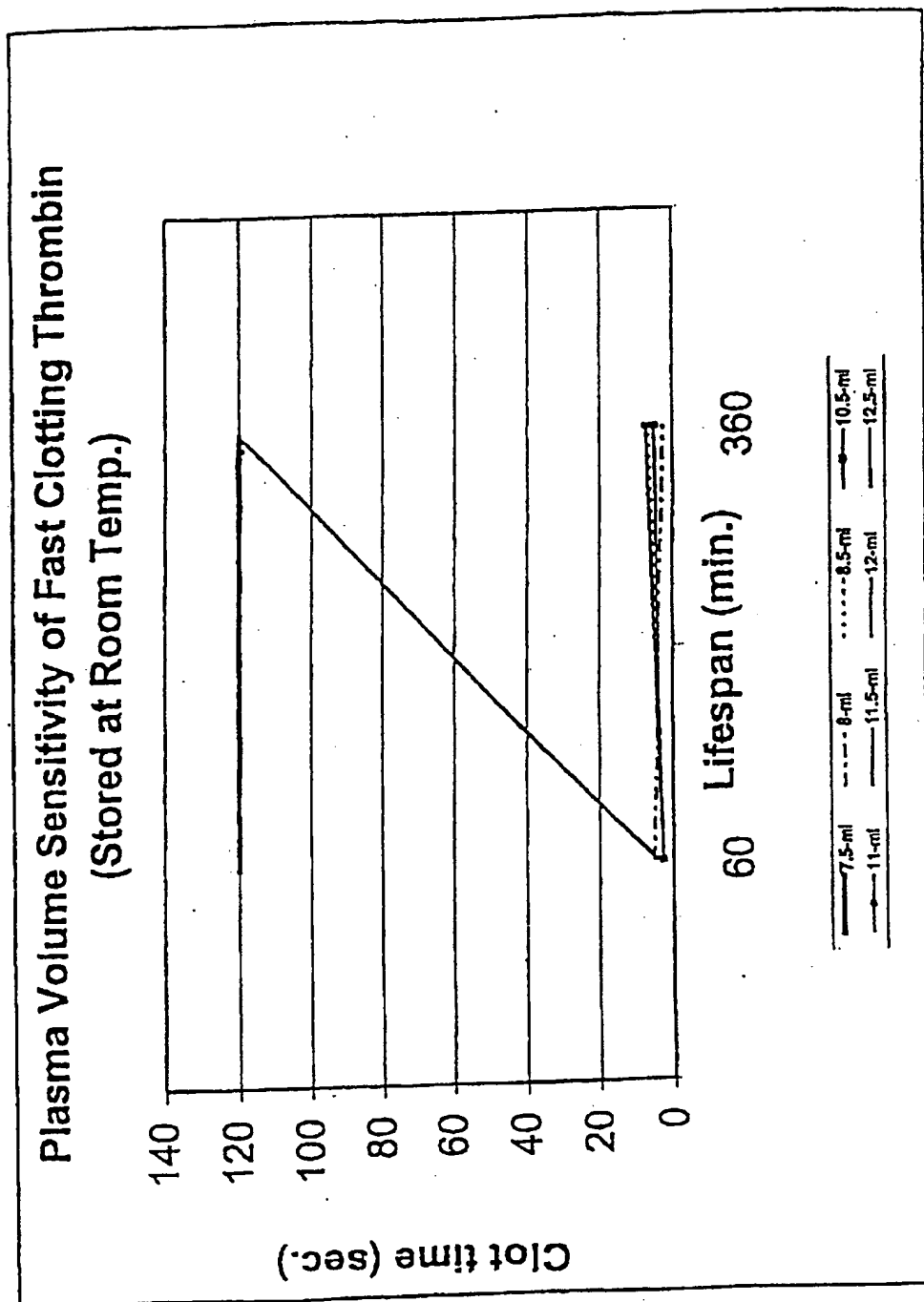


Figure 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/11865

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 38/48; B01L 11/00; C12N 9/74; C12Q 1/56; G01N 33/00

US CL :422/73, 101; 424/94.64; 435/13, 214

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/73, 101; 424/94.64; 435/13, 214

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, CHEMICAL ABSTRACTS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/31245 A1 (HAMOLTON CIVIC HOSPITALS RESEARCH DEVELOPEMENT, INC.) 10 October 1996, see page 7 first full paragraph.	1-27
X	US 5,643,192 A (HIRSH et al.) 01 July 1997, see column 4 Example.	1-27
X	US 5,795,780 A (CEDERHOLM-WILLIAMS et al.) 18 August 1998, see column 5.	1-27
A, P	WO 99/45938 A1 (BIOSURGICAL CORPORATION) 16 September 1999, see entire document.	1-27
A, P	US 6,063,297 A (ANTANAVICH et al.) 16 May 2000, see entire document.	1-27
A	US 5,510,102 A (COCHRUM) 23 April 1996, see entire document.	1-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 AUGUST 2000

Date of mailing of the international search report

28 AUG 2000

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